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INTRA-UTERINE GROWTH AND ITS RELATIONSHIP TO CONGENITAL ANOMALIES

by

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TABLE OF CONTENTS

		PAGE
I.	INTRODUCTION	1
II.	MATERIALS AND METHODS	3
III.	RESULTS	6
IV.	DISCUSSION	17
V.	SUMMARY	21
BIBLIOGRAPHY	. . . . .	22

I. INTRODUCTION

It was the observation of Owen Steir that seven infants with cleft palate were born at term but had a lower birth weight ther the "average infant" (1). Whether this was an incidental observation or a significant fact was of interest because it suggested intra-uterine growth of the fetus had been impaired, perhaps by the same factors causing the cleft palate. The following studies were undertaken to learn if intra-uterine growth retardation was associated with circumstances causing anomalies, such as cleft palate.

Within the past twenty-five years, experimental evidence has accumulated indicating that various disturbances of mammalian fetal environment will result in predictable congenital malformations. In 1935, Hale reported on the relation of vitamin A to anophthalmos and cleft palate in pigs (2). Other insults, including maternal vitamin deficiencies and excesses, x-rays, infectious disease, anoxia, injections with certain chemicals and hormones have also been shown to induce congenital anomalies (3 - 12). These workers have demonstrated a number of methods by which anomalies could be induced. For the purpose of this study, rats were chosen as the experimental animal because their fetuses are five times larger than mice, and according to the method of Woollam and Millen, cleft palates were induced in 100% of

the offspring by using excessive doses of vitamin A and cortisone (13). In the present study, an attempt is made to substantiate experimentally in rats that the congenitally deformed offspring are born at a decreased birth weight and length. In addition, the weights of a large series of infants born with cleft palates at the Grace-New Haven Community Hospital were reviewed to see if their weights were indeed significantly lower than the normal human infant.

II. MATERIALS AND METHODS

Female Wistar strain rats, without previous litter, weighing 200-350 gms. were mated overnight to males of the same strain. Exposure to males was allowed 8:00 p.m. - 9:00 a.m. only during the oestrus stage. This stage is characterized by a vaginal mucous membrane that is dry, lusterless or white and usually associated with turgescence of the small radiating folds about the vaginal aperture. The vaginal smear at this time shows only cornified cells and no leucocytes or epithelial cells. Females in heat exhibit a typical behavior which consists of hopping about intermittently and the head is shaken so that the ears quiver with a fine vibrating movement. These signs are indicative that exposure to the male at this time will result in impregnation. The morning following exposure, the female was examined for a vaginal plug (see Fig. 1 & 2) and if found, the date of the morning of exposure was considered as day one of gestation. This method was 100% reliable as an indication of impregnation. The females were kept in individual cages with wire bottoms in a well ventilated, thermostatically controlled room at 80°F. and 50% humidity. They were fed a stock diet of Purina lab chow and water ad libitum. All the animals were intubated with a #8 French plastic catheter on gestation days eight thru thirteen and

the experimental rats received 60,000 units (1.2 c.c.) of vitamin A, (Aquasol A - U. S. Vitamin Corp.), while the control group received 1.2 c.c. of arachis oil. Subcutaneous injections of 20 mgm. (.8 c.c.) of cortisone acetate were given daily to the experimental rats on gestation days nine thru twelve while the control rats received .8 c.c. of normal saline s.c. Pregnancy was allowed to proceed to term (22 days). The control animals delivered spontaneously under close observation. The experimental animals were anesthetized with ether and Caesarian sections were performed because the maternal rat will destroy grossly deformed or dead offspring if allowed to deliver spontaneously. Offspring from both groups were examined grossly for malformations and weights and lengths were taken. Each fetus was weighed on a balance scale to the nearest tenth of a gram within an hour of delivery. Lengths were measured from tip of snout to anus on a flat surface table, in millimeters. The deformed fetuses were then fixed in formalin.

The charts of 136 full-term infants born from 1920-52 with cleft palate alone or with other anomalies were reviewed and birth weights were recorded and analyzed statistically.

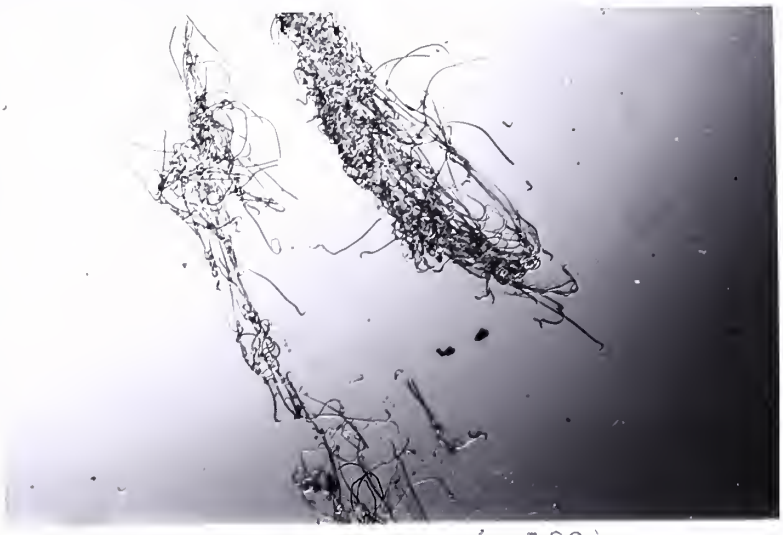


Fig. 1 (x 100)

Fig. 2 (x 400)

Fig. 1 and 2: Photograph of a slide (stained with methylene blue) of the sperm plug found in the female vagina following a successful copulation.

III. RESULTS

In the first series of experiments, the experimental rats were treated as described but with a preparation of vitamin A that evidently was not potent as none of the animals showed any signs of vitamin A effect - anorexia, dull eyes, listlessness, hemorrhage, rough fur or litter failure.

It was decided to try another preparation of vitamin A on the next series of nineteen experimental rats. In this group, only seventeen survived as two rats succumbed during intubation and instillation of vitamin A into the trachea. In this group, (Table 1) only three females carried their pregnancy to term, producing thirty-two offspring. The pregnancy rate being 3/17 or 17.6%. (Conlan reports a pregnancy rate of 12% when using only excessive vitamin A in 210 rats) (6). The eleven normal control rats had a pregnancy rate of 100%. (Conlan reported 68% in his control group.) The fourteen experimental animals not going to term exhibited vaginal bleeding during the 12-15th day of gestation and the products of conception were either aborted or reabsorbed. The experimental animals that did carry the pregnancy to term produced thirty-two offspring in which twenty-eight or 87.5% had one or more gross abnormalities; 62.5% of the total had cleft palates. Table 2 is a summary

of the anomalies found. Many offspring had multiple anomalies, such as exencephaly, macroglossia, partially fused mouths, and gross eye defects. Photographs 3, 4, and 5 are representative anomalies of the experimental group. The uteri of the experimental animals were moderately congested and the amnionic sacs of the malformed fetuses contained blood-tinged fluid. The placentas of some fetuses had a yellow cheesy surface that easily peeled off. This, however, was not a consistent finding in all the experimental rats.

Table 3 is a summary of the findings in the control animals. The average birth weight of 128 offspring was 5.9 gm. The average birth length was 51 millimeters. Table 4 is a summary of the findings in the vitamin A and cortisone treated animals. The average birth weight of twenty-eight malformed offspring was 4.2 gm. The average birth length was 42 millimeters. The decrease in these animals mean weight from the control value was $\frac{1.7}{5.9}$ equals 28.8%, and is statistically highly significant. Likewise the decrease in the experimental animals' mean birth length was $\frac{9}{51}$ equals 17.6% and is also statistically significant. Clinical data of infants born with cleft palate at Grace-New Haven Community Hospital from 1920-1952 is summarized in Tables 5 & 6. Fifty-eight females with cleft palate ranged in birth weight from 1,417 - 4,150 gms. Their

average birth weight was 3083.5 gms. Using 3232 gm. as the average birth weight for normal white females and applying the "t test", the results are t = 2.076 and P < 0.05 (14). Seventy-eight males ranged in birth weight from 2040 - 4989 gm. with an average weight of 3177.3 gm. Using 3345 gm. as the average birth weight for normal white males, and applying the "t test", the results are t = 2.506 and P < 0.05. The difference in the mean birth weight of newborns with cleft palate and the normal newborn weight is therefore statistically significant.

TABLE I

LITTER RATE, ANOMALY AND CLEFT PALATE INCIDENCE IN EXPERIMENTAL AND CONTROL GROUPS

	No. of Females Mated	No. of Litters Produced	Litter Rate %	Total No. of Offspring	No. of Malformed	% of Malformed	No. of Cleft Palates	% of Cleft Palates
Experimental	17	3	17.6	32	28	87.5%	20	62.5%
Control	12	12	100	117	0	0	0	0

Table II

SUMMARY OF GROSS ANOMALIES SEEN IN OFFSPRING OF RATS TREATED WITH VITAMIN A AT CRITICAL STAGES

Animal	Cleft Palate	Exencephaly	Microcephaly	Cephalohematoma	Macroglossia and/or Micrognathia	Partially Fused Os	One Eye	No Eyes	Exophthalmos
1	*	*			*	*		*	
2	*	*			*	*	*		
3	*		*		*	*			
4					*	*			
5	*			*		*			
6	*					*			
7	*			*	bifid	*			
8	*				*	*			*
9	*				*	*			
10	*				*	*			
11	*		*		*	*			
12	*		*			epithelial tags			
13	*				*	*		*	
14	*	*			*	*	*		*
15	*				*	*			
16	*			*	*	*			
17						*			
18	*	*		*		*		*	
19	*	*		*	*	*		*	
20	*	*		*	*	*		*	
21									
22					*	*			
23	*				*	*			
24									
25					*	*			
26	*								
27	*					*			
28									

TABLE III

SUMMARY OF BIRTH WEIGHTS AND LENGTHS OF CONTROL RATS

Animal	No. of Offspring	Total Weight of Offspring gm.	Total Length of Offspring mm.	Mean Weight	S.E. of Mean	95% Confidence Limit	Mean Length	S.E. of Mean	95% Confidence Limit
1	10	56.2	493	5.62	0.27	5.1-6.2	49.3	0.96	47.1-51.5
2	14	78.1	694	5.52	0.14	5.2-5.8	49.6	0.86	48.7-50.5
3	12	71.9	650	5.53	0.09	5.3-5.7	50.0	0.45	49.0-51.0
4	8	53.4	428	6.72	0.15	6.4-7.1	53.6	0.90	51.5-55.8
5	16	83.1	792	5.19	0.12	4.9-5.4	49.5	0.68	48.0-51.0
6	15	84.7	787	5.65	0.08	5.5-5.8	52.4	0.54	51.3-53.6
7	9	54.3	457	6.03	0.21	5.5-6.5	50.7	0.90	48.7-52.9
8	6	37.0	303	6.17	0.14	5.8-6.5	50.5	0.71	48.7-52.3
9	13	77.5	664	5.96	0.07	5.8-6.1	51.0	0.41	50.1-52.0
10	12	96.2	689	6.64	0.08	6.5-6.8	53.0	0.61	51.7-54.4
11	10	63.0	511	6.30	0.12	6.0-6.6	51.1	0.62	49.7-52.5
Total	127	745.9	6467						

Average weight 5.9 gm.
Average length 51 mm.

TABLE IV

SUMMARY OF BIRTH WEIGHTS AND LENGTHS OF EXPERIMENTAL RATS

Animal	No. of Deformed Offspring	Total weight of Malformed	Total length of Malformed	Mean Weight	S.E. of Mean	95% Confidence Limit	Mean Length	S.E. of Mean	95% Confidence Limit
B	9	33.6	378	3.44	0.14	3.1-3.8	37.5	0.83	35.7-39.3
D	12	41.3	450	3.73	0.14	3.4-4.0	42.0	0.84	40.0-44.0
E	7	42.3	347	5.27	0.08	5.1-5.5	50.0	0.21	49.4-50.5
Total	28	117.2	1175						

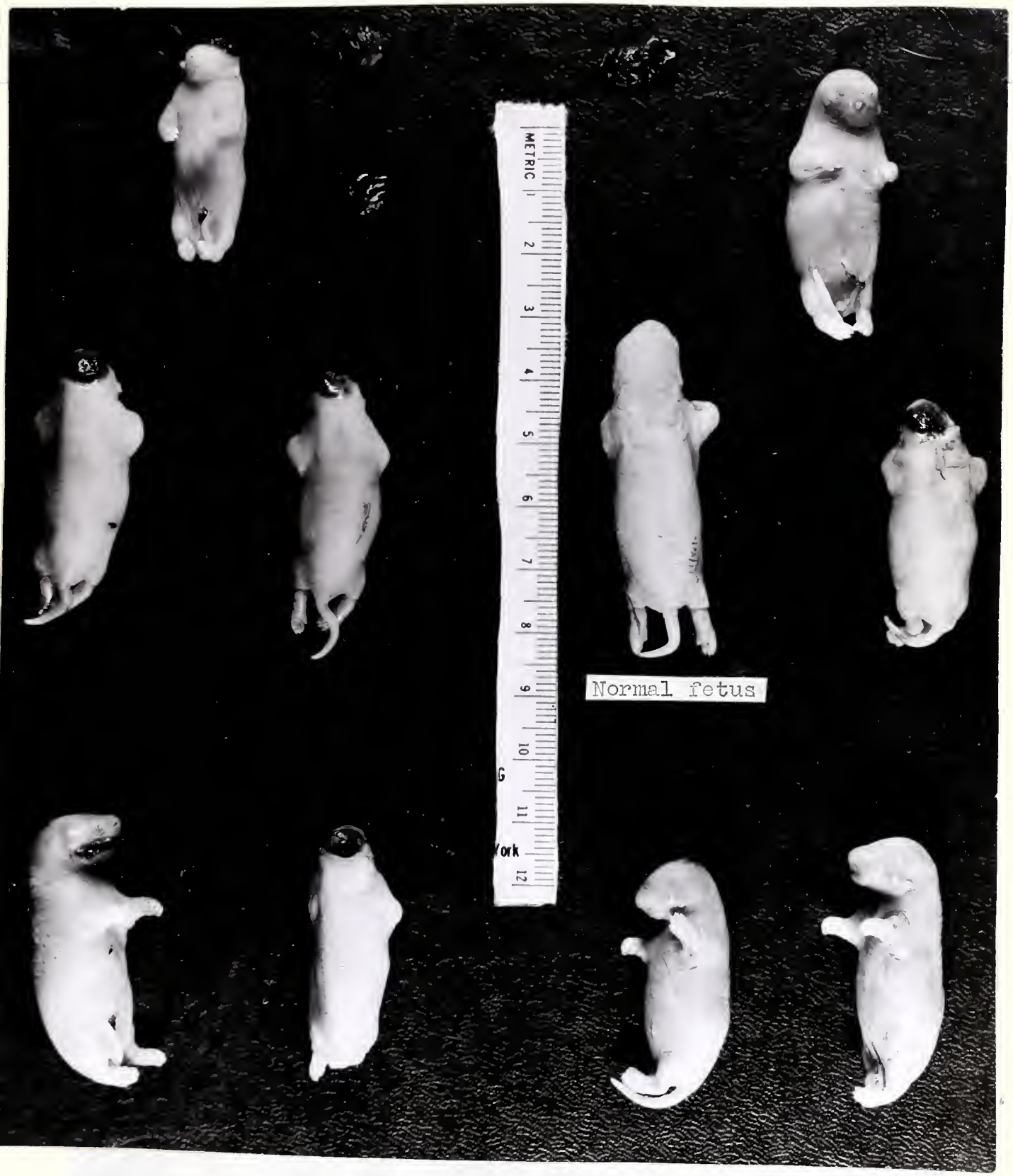
Average weight 4.2 gm.
Average length 42 mm.

TABLE V

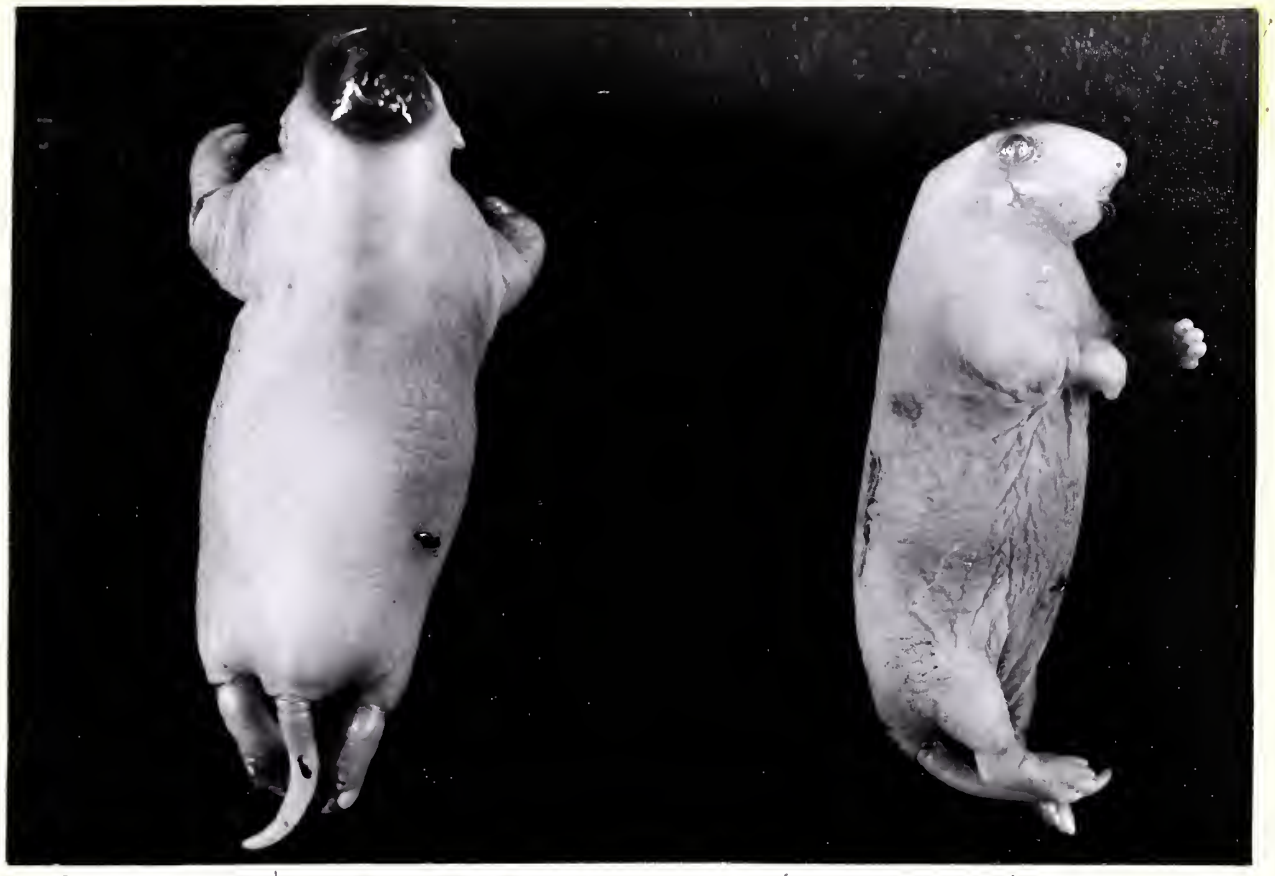
Birth Weight In Grams	2000	2000-2249	2250-2499	2500-2749	2750-2999	3000-3249	3250-3499	3500-3749	3750-3999	4000-4249	4250
No. Females	4		7	4	8	13	7	8	6	1	
No. Males		2	6	9	12	16	9	15	5	1	3
Total No.	4	2	13	13	20	29	16	23	11	2	3

TABLE VI

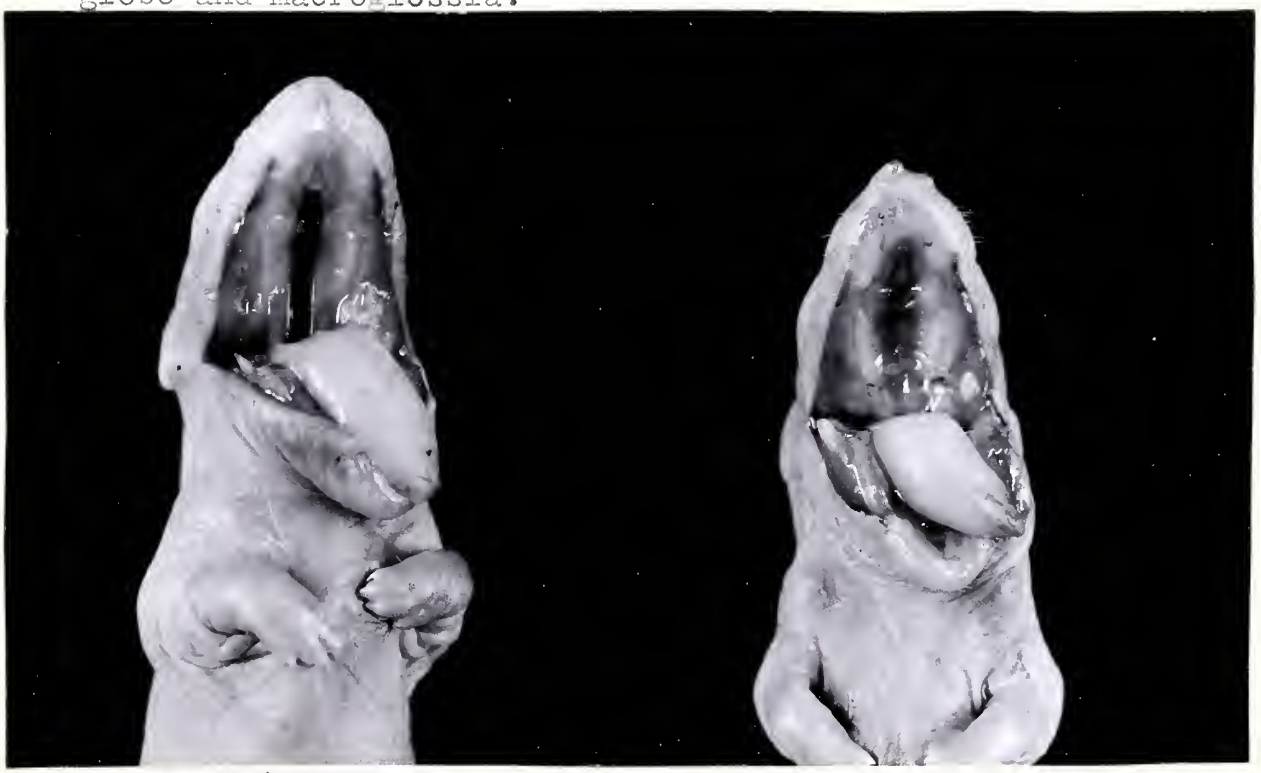
	Range of Birth Weight	Cleft Palate mean Birth Weight	Normal mean Birth Weight	Difference	"t" Value	"p"
78 Males	2040-4989 gm.	3177.3	3349	171.7	2.506	<0.05
58 Females	1417-4150 gm.	3085.5	3234	150.5	2.076	<0.05



Photograph 3: TYPICAL ABNORMAL LITTER. Maternal rat intubated with 60,000 units of vitamin A and injected with 20 mgm. of cortisone during the 7-13th days of gestation. (Enlarged x1.16) Normal fetus included in middle row, third from the left for comparison of size.



Photograph 4: Experimental fetuses (Enlarged x2)
Left: Exencephaly Right: "Open Eye" with bulging of globe and macroglossia.



Photogrph 5: Fetus with cleft palate on left. Normal palate on right. (Both mandibles have been incised for exposure) Enlarged x 4.

DISCUSSION

If birth weight of a newborn is used as one measurement of intra-uterine growth, it appears that the presence of an anomaly (cleft palate) in the neonate coexists with a reduction of intra-uterine growth. This correlation raises several questions. Are lighter embryos more prone to anomalies than heavier ones, or are anomalies and reduced weight the result of a more severe intra-uterine impact on some fetuses than on others? There appears to be no way of deciding between these and other possibilities. It has been demonstrated that an insult at a crucial developmental moment may be capable of having a major effect on the fetus. The insult of hypervitaminosis A and cortisone has demonstrable teratogenic effects on the developing embryos when injected into pregnant Wistar rats. The role that the vitamin A and cortisone play in producing the anomalies and diminished birth weight is not well understood but perhaps not unexpected in view of the demonstrated capacity of cortisone to retard skeletal growth maturation in the human (15, 16).

Woollam believes that in the rat the action of cortisone is a general one and in some way serves to sensitize the tissue to vitamin A and to potentiate the action of the vitamin in the production of malformations (17). There is no evidence that cortisone has any effect by itself as a

teratogenic agent in the rat. However, Fainstat has been able to produce cleft palate in the rabbit by administration of cortisone alone and Baxter and Peer have induced cleft palate in mice with just cortisone, Pickman has demonstrated that when cortisone is administered to chick embryos on the initial day of incubation and two or three days after, there is a retardation of growth, and he believes this growth retardation to be " in all likelihood, a specific hormonal effect." (9, 10, 16, 19). Pickman does not consider the cortisone to be an antagonist to hypophyseal Growth Hormone inasmuch as Fugo observed that hypophysectomy of the chick embryo had no effect on its growth and development until after the tenth day of incubation (20).

The experimental rats failed to achieve a normal weight by a factor of 28.8% and a normal length by 17.0%. By analogy with growth retardation in the human this finding would support the contention of Pickman that the retardation had not been the consequence of simple deprivation of growth hormone, but more likely, perhaps, the consequence of interference with normal nutrition of the fetus by either supply of adequate nutrients or utilization of nutrients.

The many effects of cortisone upon the experimental animal indicate that some basic process necessary for the health of the individual cell, and of the organism as a whole, is affected by this hormone. There are reports in the litera-

ture of a disturbance of nitrogen balance and suppression of protein synthesis and of the "depolymerization" of the ribonucleic acids of liver mitochondria and microsomes upon treatment of the animal with cortisone (21, 22). The disturbance of the nitrogen balance may be due to interference with functions of vitamin B_6, which is a coenzyme in the metabolism of amino acids. A stimulation of liver transaminase activities has been observed when rats were treated with cortisone (23). Acceleration of specific transaminase activities may cause a more rapid synthesis of certain amino acids at the expense of others, thus altering the proportions of the various amino acids available for protein synthesis (23). This may result in an interference with protein metabolism, which in turn would interfere with growth in the embryo, producing congenital anomalies. It is probable that cortisone interferes with the metabolic functions of vitamin A, B_6, folic acid and riboflavin which are essential for enzymatic processes and vital for normal differentiation. An excess of one of these, e.g. vitamin A, may act at a critical normal developmental period as an antienzyme or antimetabolite to interfere with the net rate of synthesis of proteins and nucleic acids and hence retard growth of the embryo.

It is noteworthy from a clinical standpoint that the susceptible critical period of rapid differentiation sensitive to teratogenic agents in the rat corresponds to the second

to twelfth postconceptual week in the human. Reports have appeared in the literature where individuals with cleft palate were delivered by mothers who had received cortisone therapy during early pregnancy (24, 25). Though we certainly cannot, at present, relate the observations of the teratology of cortisone and vitamin A excess in the rat to human malformations, the long-term results of experiments with cortisone and various vitamins both singly and in multiple preparations, may dictate caution in their indiscriminate use during pregnancy.

SUMMARY

(1) The administration of cortisone and excessive vitamin A to the rat during pregnancy results in offspring with multiple congenital anomalies and a reduced birth weight and length.

(2) In the review of 136 charts of children born with cleft palate, the mean birth weight is significantly lower than the "average" mean birth weight.

(3) Possible mechanisms of action of the reduced intra-uterine growth and the teratogenic effect of cortisone and excess vitamin A are discussed.

BIBLIOGRAPHY

1. Shteir, O. A.: A Study of Congenital Malformations with Special Interest in their Relation to Pelvimetry. A thesis presented to the Faculty of Yale University School of Medicine in candidacy for the degree of Doctor of Medicine. 1959

2. Hale, F.: The Relation of Vitamin A to Anophthalmos in Pigs. American Journal Ophthalmology, 18:1087 1935

3. Gilman, J., Gilbert, C., and Gilman, T.: Preliminary report on Hydrocephalus, Spina Bifida, and other Congenital Anomalies in Rats produced by Trypan Blue. South African Journal of Medicine, Sc. 13:47 1938

4. Warkany, J., and Nelson, R.C.: Skeletal Abnormalities in Offspring of Rats reared on Deficient Diets, Anat. Rec. 79:83 1941

5. Swan, C., and others: Congenital Defects in Infants following Infectious Disease, Medical Journal of Australia, 2:201 1943

6. Cohlan, S. Q.: Excessive Intake of Vitamin A as a Cause of Congenital Anomalies in Rat, Science 117:535 1953

7. Hicks, S. P.: Developmental Malformations produced by Radiation, American Journal Roentgenology, 69:272 1953

8. Warkany, J.: Congenital Malformations induced by Maternal Dietary Deficiency; Experiments and their Interpretation. Harvey Lect., 48:89 1954

9. Fainstat, T. D.: Cortisone-induced Congenital Cleft Palate in Rabbitts, Endoc. 55:502-508 1954

10. Baxter, H. and F., Fraser, G.: Production of Congenital Defects in Offspring of Female Mice treated with Cortisone. McGill Medical Journal, 19:245-249 1950

11. Ingalls, T. H.: Curley, F. J. and Prindle, R. A.: Anoxia as a Cause of Fetal Death and Congenital Defect in the Mouse, American Journal Dis. Child. 80:34-45 1950

12. Haskin, D.: Some Effects of NH_2 on the Development of External Body Form in the Fetal Rat. Anat. Rec. 493:511 1940

13. Woolam, D. H. and Millen, J. W.: Effect of Cortisone on the Incidence of Cleft Palate induced by Experimental Hypervitaminosis A. British Medical Journal ii: 196-197 1957

14. Anderson, Nina; Brown, E.; and Lyon, R.: Causes of Prematurity III. Influence of Race and Sex on Duration of Gestation and Weight at Birth, American Journal of Dis. of Child. 65:523-534 1943

15. Blodgett, F. M.; Burgin, L.; Iezzoni, D.; Gribetz, D.; Talbot, N.: Effects of Prolonged Cortisone Therapy on the Statural Growth, Skeletal Maturation and Metabolic Status of Children, New England Journal of Medicine 254:636-641 1956

16. VanMetre, T.; Pinkerton, H.: Growth Suppression in Asthmatic Children receiving Prolonged Therapy with Prednisone and Methylpresnisolone. Journal of Allergy 30:102-112 1959

17. Woollam, D.H.: The Experimental Approach to the Problem of Congenital Malformations, Ann. of the Royal Coll. of Surg. of Eng. 22:401-415 1958

18. Peer, L.A.; Bryan, W.; Strean, L.; Walker, J.; Bernhard, W.; and Peck, G.: Induction of Cleft Palate in Mice by Cortisone and its Reduction by Vitamins. The Journal of the Internat. Coll. of Surg. 30:249-254 1958

19. Pickman, D.; Ridley, A.; Orgel, M., and Blumenthal, H.: Effect of Growth of Chick Embryos during Early Embryogenesis. Endoc. 64:790-794 May 1959

20. Fugo, H.W.: Effects of Hypophysectomy in the Chick Embryo. Journal Exp. Zool. 85:271 1940

21. Allbright, F.: Harvey Lectures 38:123 1943

22. Greenberg, G.R.: Role of Folic Acid in Purine Synthesis. Fed. Proc. 13:745-749 1954

23. Brin, M. and McKee: Effects of X-Irrodiation. Nitrogen Mustard, Fasting, Cortisone and Adrenalectomy on Transaminase Activity in the Rat. Arch. Biochem. Biophys. 61:384-389 1956

24. Harris, J. and Ross, I. P.: Cortisone Therapy in Early

Pregnancy; Relation to Cleft Palate. Lancet 1:1045-1047 1955

25. Doig, R. K. and Coltman, C.: Cleft Palate following Cortisone Therapy in Early pregnancy. Lancet 2:730 1956

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